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13. ABSTRACT (Maximum 200 Words) BK virus (BKV) is a human polyomavirus that establishes a lifelong, persistent infection of the urinary tract. The virus encodes oncoproteins that have been shown to cause cancer in animal models, and recently BKV DNA has been detected in urinary tract tumors including the prostate. Prostate tumors have a relatively low frequency of mutations in the <i>p53</i> and <i>Rb1</i> genes, indicating that an outside agent such as a virus may be inactivating the function of their gene products. The aims of this proposal are to determine if BKV is present in any prostate tumors and, if so, whether viral oncogenes are expressed and deregulate cell growth control. To accomplish this, matched normal and tumor tissue from individual patients will be analyzed. PCR and in situ PCR will be performed to determine the presence of viral sequences, and RT-PCR and immunohistochemistry will be used to examine gene expression. Viral sequences from patients will be cloned and their function compared to wild type strains of the virus. During the past year, we have been refining the assays and have begun to analyze a small set of samples. The results to date are not conclusive. If BKV is associated with a subset of prostate cancers, our knowledge of the virus will be useful in designing drugs and vaccines for treatment.				
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INTRODUCTION

BK virus (BKV) is a human polyomavirus that establishes a lifelong, persistent infection of the urinary tract. The virus encodes two oncoproteins, the large T and small t antigens, that have been shown to cause cancer in animal models, and recently BKV DNA has been detected in various urinary tract tumors including tumors of the prostate. Among the spectrum of human cancers, prostate tumors have a relatively low frequency of mutations in the *p53* and *RB1* genes, indicating that an outside agent such as a virus may be inactivating the function of their gene products. Both large T and small t antigens are capable of doing so. The aims of this proposal are to determine if BKV is indeed present in these tumors and, if so, whether these oncogenic proteins are expressed and function to deregulate cell growth control.

BODY

All of our work during the first year of this project has been focussed on developing the techniques required for these analyses. Our progress is described below after the appropriate items from the approved Statement of Work

Task 1. To examine prostate tumors and matched normal tissue for the presence of BKV sequences (months 1-30)

- ‡ isolate DNA and RNA from microdissected normal and tumor tissue (months 1-6)
- ‡ perform PCR analysis of DNA (months 3-18)

We have begun these analyses as described in the proposal. The results have been difficult to interpret as of yet, however, due to a high number of false positive signals from the solution PCR. We believe that this is due to the large number of cycles which must be performed to obtain a signal (usually 50-60). We are addressing this problem in three ways. First, we have placed more emphasis on in situ assays, as described below, since this technique is less prone to false positives. Second, we are designing new primer pairs which we believe may help by amplifying larger segments of viral DNA: investigators studying the possible relationship of another polyomavirus, SV40, to human cancer have found this useful. Third, we have begun to develop immunohistochemical assays for large T antigen, which has been moderately successful (see below).

- ‡ perform IS-PCR analysis of DNA (months 3-24)

Prior to commencing IS-PCR, we decided to attempt basic in situ hybridization to detect mRNA in the cells. We have constructed a series of subclones of BKV for producing RNA probes, and have also made a subclone of the PSA gene as a positive control. To date, we have not been able to obtain a positive signal with any of the probes. We are troubleshooting these assays and hope to have the conditions worked out in a timely manner. We fully expect to see a signal with the

positive control PSA probe, as well as with the BKV probe since we are able to see T antigen protein expression by immunohistochemistry. We also will begin the straight IS-PCR studies.

‡ perform sequence analysis of DNA (months 6-24)

‡ perform RT-PCR and IS-RT-PCR analysis of RNA (months 12-30)

These parts of this task have not yet commenced.

While not listed as an original part of this task, we have explored immunohistochemistry for T antigen as an alternative approach to examine viral gene expression. We worked out the conditions for staining by growing T antigen-expressing cells on cover slips. We tested six different monoclonal anti-T antigen antibodies, and found one that reproducibly gave a signal on T antigen-expressing cells but not cells without T antigen. Moreover, under these same conditions a control monoclonal antibody gave no signal. We went on the test tissue samples. In about $\frac{1}{3}$ of the samples, we can detect T antigen-positive cells. What is difficult to explain, however, is that only a fraction of the cells in each tissue slice are positive. One of the important lessons of this first year of work has been how interdependent all these approaches are. For example, if we can get in situ PCR and RT-PCR to work, they might confirm these results.

Task 2. To determine how alterations in viral sequences in tumors affect the replication and transformation properties of the virus (months 9-36)

‡ construct genomic viral clones containing patient-derived sequences (months 9-24)

We have not yet cloned out any patient derived sequences. Such cloning requires that we are sure that the PCR assays are functioning properly. We have, however, constructed a new wild type expression vector that will facilitate cloning of the patient samples.

‡ determine the ability of these clones to replicate in permissive host cells (months 12-36)

‡ construct early region expression vectors containing patient-derived sequences (months 9-24)

‡ determine the ability of these clones to interfere with normal growth control pathways involving pRb and p53 (months 18-36)

KEY RESEARCH ACCOMPLISHMENTS

- development of solution PCR assay for BKV sequences in microdissected tissue
- development and optimization of immunohistochemical staining procedures for BKV T antigen in human tissue
- detection of BKV T antigen in human prostate tissue

REPORTABLE OUTCOMES

None

CONCLUSIONS

We have made substantial progress in developing the technical assays needed to complete the work we have proposed. Some of these assays need further work while others are running smoothly. In addition, we have begun using an approach that we hadn't proposed but complements the ones we have proposed, immunohistochemical staining, to detect viral proteins in the tissue samples. We believe that during the current year we will be able to collect meaningful data regarding the presence of the virus in human prostate.

REFERENCES

none

APPENDICES

none